Preclinical Case Studies of Gonadal Distribution

Andra Miller
FDA, CBER
3-12-99

Preclinical Case Studies of Gonadal Distribution

Gonadal distribution studies in support of initiation of gene therapy clinical trials.

- Identifiers removed
- In vivo vector administration
- Assess distribution of vector to gonads,
  not germline integration

Preclinical Case Studies of Gonadal Distribution

- Examples of data in support of phase I trials
- Discussing cases that illustrate problems and questions.
- Many cases:

  adequate assay ⇨ negative results ⇨ proceed without delay

- Insight into FDAs decision making process

Preclinical Case Studies

- Preclinical animal study issues
- Choice of detection assay
- PCR assay issues
  - Current recommendation (100 copies/μg or less)
- Disease severity and reproductive status influenced decision on clinical trial

- **Vector Class:** Plasmid (naked DNA)
- **Route of Administration:** Intramuscular
- **Disease Severity:** serious
- **PCR Assay Sensitivity:** 10 million copies/μg genomic DNA
- **Initial Assay Results:** signal in gonads below level of detection (< 10 million copies/μg)
- **Impact of review on clinical trial:** Assay sensitivity was inadequate to assess safety. Clinical trial restricted to sterile patients, pending further assay development.

Assay Amendments and Outcome: plasmid (naked DNA)

- **New PCR Assay Sensitivity:** 25 - 100 copies/μg genomic DNA
- **Results:**

	Gonads	Injected muscle
Day 3	**1/3**	**3/3**
Day 14	**0/3**	**2/3**
Saline Control (Day 3)	**0/3**	**1/3**

- Improved assay sensitivity allowed for safety assessment. Transient signal in gonads gone by day 14.
- Clinical trial allowed to proceed in fertile population.
- Positive in the control arm indicates possible problem with contamination.

- **Vector Class:** Plasmid +lipid

- **Route of Administration:** Intraperitoneal

- **Disease Severity:** Life threatening

- **PCR Assay Sensitivity:** 2000 copies/μg genomic DNA

Initial Assay Results: plasmid + lipid

	Ovary	Vehicle control
4 hours	**5/5***	**0/5**
3 days	**5/5***	**N/A**
7 days	**5/5***	**N/A**
14 days	**5/5***	**N/A**

Impact of review on clinical trial:

- Despite positive signal in gonads and inadequate assay sensitivity, clinical trial was allowed to proceed due to disease severity, and judgement that disease precluded reproduction in this patient population.
- Informed consent document was modified to include a discussion of the preclinical findings.
- **Decision relevant for this indication only**, because of disease severity and reproductive status.
- Duration of positive signal not assessed.

Assay Amendments and Outcome: plasmid + lipid

- **New PCR Assay Sensitivity:** 330 copies/μg genomic DNA

- **Results:**

1) Intraperitoneal

	Ovary	Testis	Control
Day 8	**3/3**	**3/3**	**0/6 (M&F)**
Day 15	**3/3**	**3/3**	**N/A**

2) Subcutaneous - Day 2 results

	Gonad	Injection site	Control
Male	**0/3**	**1/3**	**0/1**
Female	**0/3**	**3/3**	**0/1**

- New study data supported new route of administration/indication.
- Today in general, assay sensitivity would not be adequate.
- Both life threatening indications, severity **and** reproductive status considered.

- **Vector Class:** Adenoviral

- **Route of Administration:** systemic

- **Disease Severity:** serious

- **Initial Assay Results:** Gonads not assayed

- **Impact of review on clinical trial :** Safety of gonadal distribution couldn't be assessed. Clinical trial restricted to sterile patients.

Assay Amendments and Outcome: Adenoviral

- **New PCR Assay Sensitivity:** 2,000 copies/μg (ovary)
  20,000 copies/μg (testis)

	Ovary	Testis	Control
Day 5	**0/1**	**1/1**	**0/2 (M&F)**
Week 4	**0/1**	**0/1**	**0/1 (M only)**
Week 12	**0/1**	**0/1**	**0/1 (M only)**

Large animal model

- Restriction to sterile patients continued.
- Data did not alleviate concern for dissemination of vector, due to inadequate assay sensitivity and small sample size.
- Sponsor carrying out further analysis

- **Vector Class:** Adenoviral (CAT vector)
- **Route of Administration:** Intramuscular
- **Disease Severity:** serious
- **CAT Assay**: Gene expression

- **Initial Assay Results:** **Adenoviral (CAT vector)**

  Day 7: gonads negative for CAT activity

- **Impact of review on clinical trial:**

  -Clinical trial proceeded without restriction. PCR studies are currently underway.

  -Today these data would not support initiation of clinical trial.

  -Inappropriate assay performed. Expression assay does not assure absence of vector sequences in gonads

Vector Class: Retroviral

- **Route of Administration:** Intratracheal
- **Disease Severity:** Life threatening
- **PCR Assay Sensitivity:** 1 copy/μg genomic DNA
- **Initial Assay Results:** **Day 30 | Ovary | 0/2**
- **Impact of results on clinical trial:** Proceeded without restriction.
- Would not support initiation of clinical trial today.
- Doesn't support conduct of trial in both male and female
- Inadequate number of animals included in study
- No assay controls

Preclinical Case Studies

- **Route of administration influences distribution.**
  (vector modifications)
- **Preclinical studies must have adequate sensitivity, specificity, and duration to assess vector localization.**
- **Presence of vector rather than gene expression should be measured.**
- **Positive gonadal signal ⇨ Risk of a germline event?**